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Formation and Structure of Compression Wood Tracheids Induced by Repeated Inclination in *Taxus cuspidata* [*1]

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繰り返し傾斜刺激を受けたイチイ材における圧縮あて材形成と仮道管の構造変化 [*1]

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仮道管の内壁にラセン肥厚をもつイチイの苗木を用い，樹幹に繰り返し傾斜刺激を与え，圧縮あて材の形成状態と仮道管の壁構造の変化を観察した。圧縮あて材は傾斜刺激に対応して形成され，その割合は傾斜期間に比例して増加した。圧縮あて材仮道管は1日約1個形成され，その分裂頻度は正常材仮道管の約3倍であった。また，傾斜刺激に伴う仮道管の壁構造の変化は次のように進行した。圧縮あて材の出現過程では，(1)ラセン肥厚の回旋方向は S → Flat → Z の順に変化し，その移行初期に，(2)S_3 が欠落し，その後，(3)S_2(L)が出現した。消失過程では，(1)ラセン肥厚の回旋方向は Z → Flat → S の順に変化し，(2)S_3 が復活，その後，(3)S_2(L)が消失した。また，圧縮あて材出現過程の移行域で，通常の圧縮あて材仮道管に見られるリグニン分布とは異なり，S_2 層の内側に木化の程度の強い層をもつ仮道管が観察された。このことは仮道管の壁形成に伴う壁成分の堆積過程と関連し，興味深い。これらの結果に基づき，正常材と圧縮あて材におけるラセン肥厚の性質について考察した。

The formation and structure of compression wood tracheids in the stems of ichi (*Taxus cuspidata* Sieb. et Zucc.) subjected to alternating positioning from vertical to inclined positions, and *vice versa*, were examined.

Compression wood arcs were formed corresponding to the changes in the stimulus of inclination, and the number of compression cells formed increased with the extension of the inclination period. The number of compression and normal wood cells formed per day was about 1 and 0.3, respectively.

Changes of cell wall structure in the transition from normal to compression wood are summarized as (1) the turn from an S- to a Z-helix of helical thickenings, (2) the disappearance of the S3 layer, and then, (3) the appearance of an S2(L) layer. On the other hand, the changes in the reverse transition are (1) the turn from a Z- to an S-helix of helical thickenings, (2) the restoration of the S3 layer, and then, (3) the extinction of the S2(L) layer. Such changes of the cell wall structure in the transition between normal and compression wood were observed alternatively and corresponded to the changes of the stimulus of positioning. Unusual cells with a lignin-rich layer in the inner region of the S2 layer were observed. These always appeared at the beginning of the transition from normal to compression wood, an interesting fact in correlation with the development of the cell wall and the deposition process of wall components.

The nature of helical thickenings in normal and compression wood is discussed.

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1. INTRODUCTION

It has been thought that reaction wood acts to maintain or restore a genetically determined position of each point of a tree inclined by a certain environmental factor[1)2)]. Compression wood usually is formed in the lower portion of inclined stems or branches. Compression wood tracheids have peculiar cell wall structures, quite different from those of normal wood tracheids. Compression wood tracheids are identified by the rounded outline in cross-section, a relatively thick S2 layer with helical cavities, the presence of an S2(L) layer, and the absence of an S3 layer. However, fundamental factors differentiating unusual tracheids and the functional significance involved in compression wood cells remain unexplained.

Although the cell wall structure, typical of compression wood in various gymnosperms, has been investigated extensively, the transitional tracheids between normal and compression wood were treated only in a few studies[3)]. In more recent reports[4)5)], the cell wall structure of such tracheids was studied in detail. However, studies on the transition of the cell wall structure between normal and compression wood are very few, for example, Takaoka and Ishida[6)], regarding softwoods species with helical thickenings on the inner surfaces of normal tracheids.

Results of investigations on the nature of helical thickenings of normal and compression wood also were inconsistent. Wardrop and Dadswell[7)] found that in *Pseudotsuga* and *Taxus* the orientation of helical thickenings paralleled the microfibrils of the S2 layer and changed with cell length. Patel[8)] suggested that helical thickenings of *Taxus* and *Torreya*, in both normal and compression wood tracheids, may indicate the microfibrillar angle in the secondary wall layer on which such thickenings are laid down, regardless of the cell width and the wall thickness. Later, Timell[9)] concluded that the thickenings are an integral part of the S3 layer in normal wood and of the S2 layer in compression wood because they have the same orientation as the innermost microfibrils in these layers. He stated that the helical thickenings and cavities seem to be mutually exclusive in compression wood of *Pseudotsuga* and *Taxus*. On the other hand, Takaoka and Ishida[6)] observed the thickenings to be laid on the S2 layer with helical ridges in late wood tracheids of *Pseudotsuga*, and this observation was confirmed later by present authors[10)].

In the previous investigation[11)], it was observed that the direction of the spirals changed gradually from an S-helix to a Z-helix in the transition from normal to compression wood, and from a Z-helix to an S-helix in the reverse transition zone, corresponding to the changes of the stimulus of inclination from a vertical to an inclined position, and *vice versa*, in *Taxus* and *Torreya*.

According to Frey-Wyssling[12)], the origin of the forces leading to the bending of a tree exists in the cambium. It is of interest to note the response of the differentiating cells to the stimulus of inclination in order to clarify the mechanism of compression wood formation. Therefor, the present investigation is focused on the formation and structure of compression wood tracheids in a stem of ichii, *Taxus cuspidata* (Sieb. et Zucc.), subjected to alternating vertical and inclined positions. On the basis of results obtained in addition to previous observation, the characteristics of helical thickenings in normal and compression wood are discussed.

2. MATERIALS AND METHODS

On May 1, 1982, three young ichii trees growing in the nursery of Utsunomiya University were inclined and fixed at 1 m height above ground at an angle of about 60° from the vertical. After the three trees were subjected to inclination periods of 5, 10, or 20 days, they were returned to a normal vertical position. After each sample tree was maintained vertically for 20 days, the second inclination treatment was made and again was followed by 20 days in the vertical position. These cycles were continued until the trees were cut on October 21.

Small wood-blocks were cut from the stems at a height of 20 cm and immediately fixed with 2% glutaraldehyde. Transverse sections 4 μm thick were sliced from these blocks and were observed under polarizing and fluorescence microscopes without staining to determine the presence of an S3 layer and the distribution of lignin in the S2 layer. The radial surfaces of inner tracheid walls were observed with a scanning electron-microscope(SEM).

Microfibrillar orientation in the S2 layer was

determined by the angle to the cell axis of elongated crystals of iodine brought out in the interstices of the S2 layer by the method of Kobayashi[13] using 20 μm thick radial sections under a Nomarski differential interference microscope. After washing and dehydrating, the same sections were observed by a scanning electron-microscope to determine the orientation of the helical thickenings.

3. RESULTS AND DISCUSSION

3.1 Formation of compression wood tracheids

Several compression wood arcs were formed in the lower side of the stems subjected to the alternating positioning. Fluorescence micrographs of transverse sections of sample trees are shown in Fig. 1. It is evident that compression arcs, recognized as bands of heavily lignified cells under a fluorescence microscope, are formed corresponding to each stimulus of positioning.

Radial cell counts for each sample tree are shown in Table 1. Heavily lignified cells with an S2(L) layer in the S2 layer included in each arc were counted as compression wood tracheids, and the intervening cells without an S2(L) layer in the S2 layer between arcs were counted as normal wood tracheids. The average numbers of compression and normal wood cells formed within and between arcs were 4.5 and 5.5, respectively, in Sample-tree (1). Corresponding figures for Sample-tree (2) were 9.3 and 6.8, respectively, and 20.3 and 6.7, respectively, for Sample-tree (3). The intervening cells formed between arcs were almost constant in each sample tree. Sample-tree (1), given 5-day periods of inclination, had a smaller number of compression cells than did the other two sample trees. The number of compression wood tracheids formed increased with extended inclination periods. The number of compression and normal tracheids formed per day is shown in Table 2. From the reciprocal of the number of compression wood cells formed per day in each cycle, about one new compression tracheid was derived each day in each sample tree in this experiment, a number nearly in accord with results of an earlier report[3]. Conversely, about 3 days were required for the derivation of one normal tracheid. Hence, the rate of cell division in the lower side of each stem, when trees were inclined to about 60°, was about three times that in normal wood formation when in the vertical position.

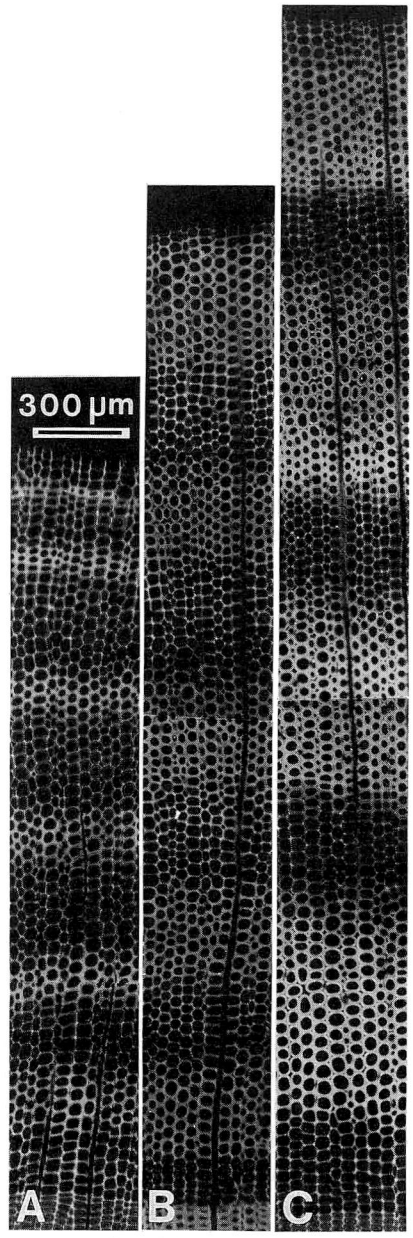

Fig. 1. Several compression wood arcs formed in the lower side of the stems subjected to the alternating positioning. A : Sample-tree (1), B : Sample-tree (2), C : Sample-tree (3).
Note : Compression arcs are recognized as bands of heavily lignified cells under a fluorescence microscope.

3.2 Transition of tracheid wall structure between normal and compression wood

A fluorescence and a polarizing micrograph of

Table 1. Number of compression and normal tracheids formed at a height of 20 cm in stems subjected to alternating positioning.

Sample tree	Inclination angle (degrees)	Inclination periods (days)	Vertical periods (days)	Cell type	Compression arc no. 1	2	3	4	Total	Ave.
1	60	5	20	C[a)]	5	4	5	4	18	4.5
				N[b)]	4	6	6	6	22	5.5
2	60	10	20	C	8	9	11	9	37	9.3
				N	6	6	8	7	27	6.8
3	60	20	20	C	18	22	21	—	61	20.3
				N	6	8	6	—	20	6.7

a) Compression tracheids with S2 (L) layer in the S2 layer.
b) Normal tracheids.

Tadle 2. Number of tracheids formed per day.

Sample tree	Cell type	Compression arc no. 1	2	3	4
1	C[a)]	1.0	0.8	1.0	0.8
	N[b)]	0.2	0.3	0.3	0.3
2	C	0.8	0.9	1.1	0.9
	N	0.3	0.3	0.4	0.4
3	C	0.9	1.1	1.1	—
	N	0.3	0.4	0.3	—

a) Compression wood tracheid.
b) Normal wood tracheid.

transverse sections of the first compression-arc formed in Sample-tree (1) given 5-day periods of inclination are shown in Figs. 2 A and B, respectively. The cross-sectional transition of the cell wall structure between normal and compression wood is in good agreement with the results of earlier investigations[4)5)].

The disappearance of the S3 layer was detected in Cell (6). Then the cell wall thickness gradually increased from Cell (6) to Cell (9). The cross-sectional shape of the cells started to be slightly rounded at Cell (6). Conversely, the outer region of the S2 layer also was faintly rich in lignin in the same cell. The width and intensity of lignification of the S2(L) layer gradually increased from Cell (6) to Cell (8) with an increase of wall thickness and roundness. Then the intensity of the S2(L) layer gradually decreased from the Cell (8) to Cell (10). The first restoration of the S3 layer occurred in Cell (10) which had a faint S2(L) layer at the cell corner. During the transition from compression to normal wood, the S2(L) layer gradually disappeared. The rapid decrease of cell wall thickness could not be seen

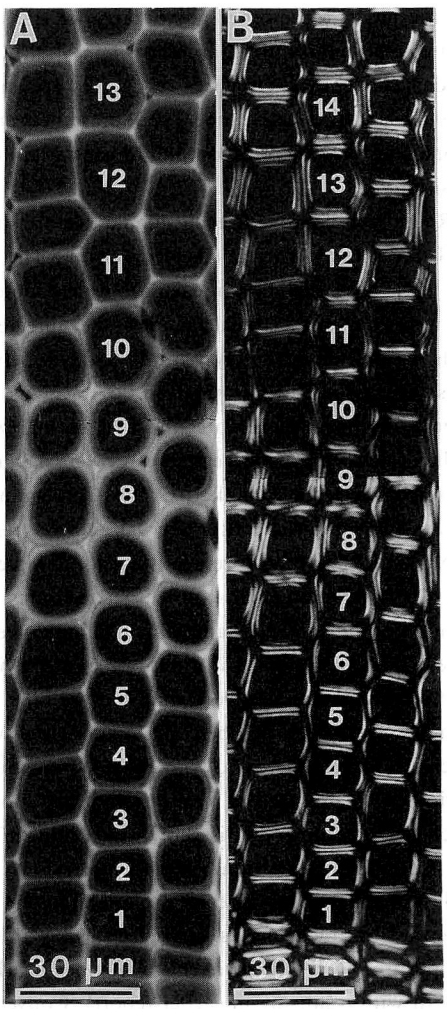

Fig. 2. The cross-sectional transition between normal and compression wood in the first compression-arc of Sample-tree (1). A: Fluorescence micrograph, B: Polarizing micrograph.

after the deposition of the complete S3 layer.

Similar cross-sectional transitions of cell wall structures also were seen in the succeeding compression arcs except for the appearance of an unusual lignin-rich zone in the inner region of the S2 layer. Fig. 3 shows the transition of the cell wall structure

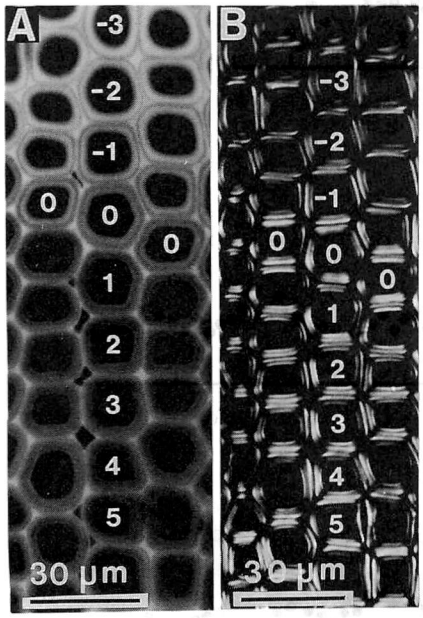

Fig. 3. The cross-sectional transition at the beginning of the second compression-arc formed in Sample-tree (3). A : Fluorescence micrograph, B : Polarizing micrograph.
Note : Cell (0) has an unusual lignin-rich layer in the inner region of the S2 layer.

from normal to compression wood in the second compression-arc formed in the sample tree given 20-day periods of inclination. In the first transition from normal to compression wood, the first excessive lignification was observed in the outer region of the S2 layer at the cell corner. However, before the appearance of compression cells with an usual S2(L) layer, some cells having an unusual distribution of lignin inside of the S2 layer appeared.

Although it was reported that the S3 layer has a greater lignin concentration than that in other layers of the secondary wall, especially in the late wood of juvenile wood[14)15)], the S3 layer was absent in Cells (0). The width and intensity of the lignin-rich zones in these cells differed from those of Cells (−1)−(−3). Also, the secondary wall showed considerable birefringence under a polarizing microscope using crossed Nicols. The birefringence of the S2 layer was greater than that of usual compression wood cells indicating that in this layer there was a relatively larger fibril-angle than in usual compression cells. This unusual lignin-rich zone spread outwards and finally developed into the S2(L) layer typical of compression wood tracheids, the width and intensity of which were greater than those of compression Cells (7)−(9) formed in the first arc.

Yumoto[5)] reported the same result using a SEM-UVM combination method and noted that an unusual ultraviolet(UV) absorption in the inner region is seen in late wood. In our experiment, such cells with unusual lignin distribution always appeared at the beginning of compression arcs formed by each period of inclination, except for the first arc. This is interesting in relationship to the response of differentiating cells to the stimulus of inclination. Such a lignin deposition suggests that tracheids in the S2-depositing stage had stored considerable lignin precursors before inclination[4)], and after inclination, as the S2-deposition stopped and lignification proceeded, the surplus lignin is incorporated into the inner region of the S2 layer.

Because lignin seems to be deposited in the inner region of the secondary wall more than in the outer region during the late stage of lignification as suggested by Takabe[16)], it is conjectured that an additional lignin deposition takes place in the inner region of the S2 layer. Dense lignin deposition inside the S2 layer never was recognized in the transition zone of normal to compression wood of the first arc of each sample tree. The cause of the difference of lignification between the first and the other arcs having various responses by the differentiating cells to the stimulus of inclination is unknown, but it seems to be related to the elongated period required for lignin deposition because of the increase of cell wall thickness.

The changes in the cell wall thickness and the cell radial diameter within an annual ring of each sample tree are shown in Fig. 4. A decrease in cell wall thickness was found in the tracheids immediately before the first appearance of an S2(L) layer in the S2 layer, as indicated by double arrow heads in Fig. 4. Similarly, it often was observed that the radial di-

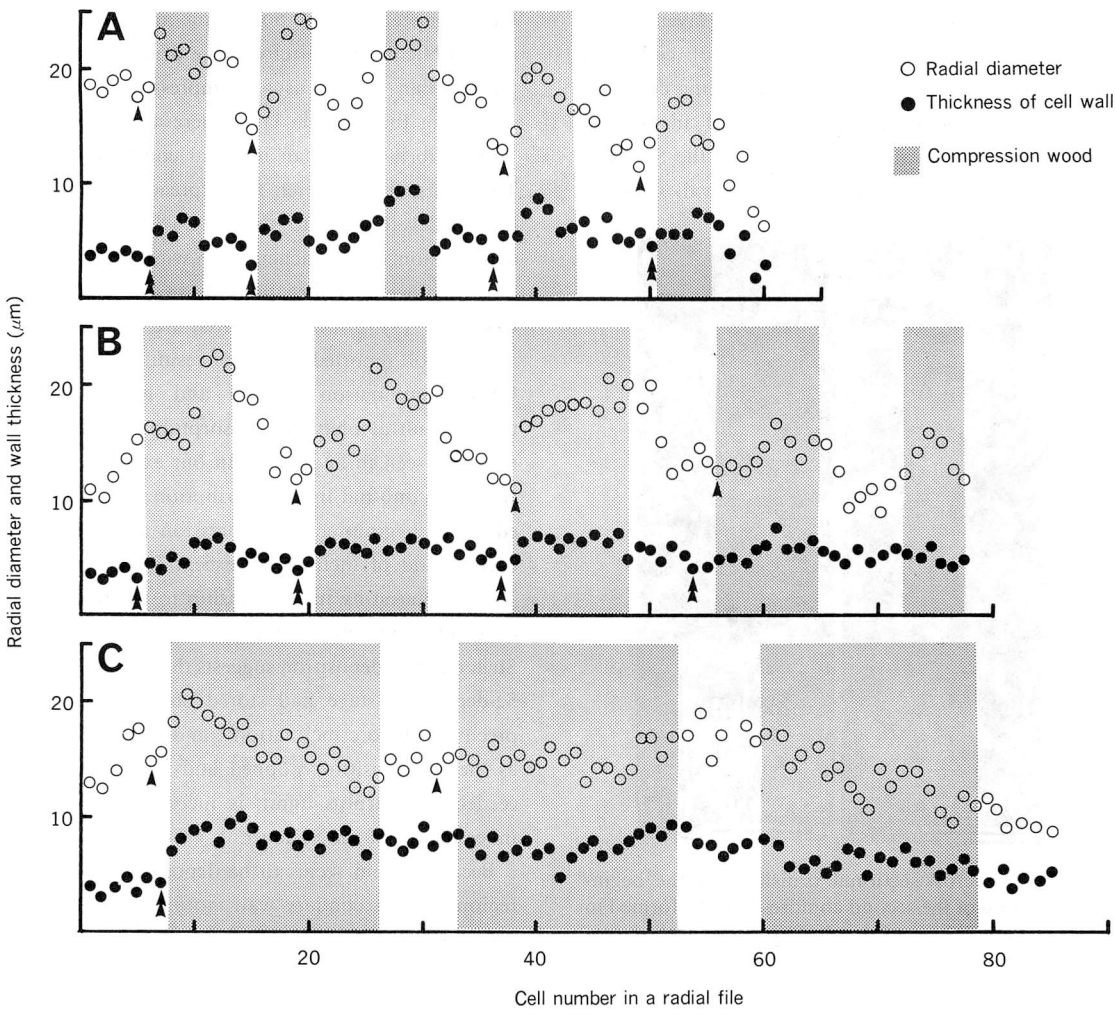

Fig. 4. Changes of the radial diameter and cell wall thickness within an annual ring. A : Sample tree (1), B : Sample tree (2), C : Sample tree (3). Single and double arrow heads indicate temporal decreases in cell radial diameter and wall thickness, respectively.

ameters of tracheids decreased temporally at the beginning of compression arcs, as indicated by single arrow heads. It was reported that the decrease of the wall thickness was caused by the lack of the S3 layer[4]. However, apparently this is related to the decrease in the thickness of the S2 layer as suggested by Yumoto[5]. The changes of both cell wall thickness and cell diameter within an annual ring, induced by the alternating periods of vertical and inclined positions, were notable in Sample-tree (1). In the other sample trees, however, the variation in cell wall thickness became relatively smaller. No appreciable difference in cell wall thickness between intervening and compression cells was found in Sample-tree (3). This suggests that the fairly gradual decrease in cell wall thickness might occur in the disappearing process of compression wood. Although the cause is unknown, a latent stimulus in the cellular level induced by the extended inclined periods exists in the differentiating zone even after the return from the inclined position.

A scanning electron-micrograph of the transition zone between normal and compression wood in a radial section is shown in Fig. 5. In normal wood

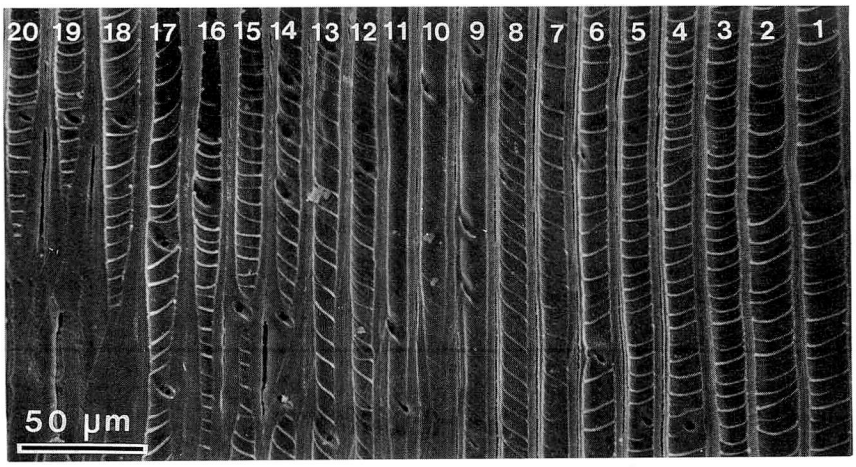

Fig. 5. A scanning electron-micrograph of the inner surfaces of the tracheid transition between normal and compression wood.

tracheids, helical thickenings were attached to the S3 layer appearing as an S-helix in parallel with the microfibrils of the S3 layer, whereas they were oriented as a Z-helix in compression wood tracheids where the S3 layer was absent and no helical cavities occurred. The direction of the spirals gradually changed from an S-helix to a Z-helix, or from a Z-helix to an S-helix, during the transition between normal and compression wood. The lack of an S3 layer was detected at Cell (7) in this figure, and the restoration of the S3 layer was found in Cell (17). The disappearance or restoration of the S3 layer seems to occur simultaneously with or immediately after the beginning of the turn of the spirals in both transitions. These successive changes of cell wall structure, which coincided well with a previous observation[11], could be observed in all of the compression arcs formed by each tilting cycle.

Fig. 6 reveals that the variation in the fibrillar angle of the S2 layer corresponds to the changes of the stimulus of inclination, as well as the shifting of the orientation of the thickenings. The modified S2 layer in compression wood tracheids had a greater microfibrillar angle than the intervening tracheids between compression arcs. In addition, the fibrillar angle of the S2 layer of these intervening tracheids was greater than that of the early wood tracheids in the beginning of the growth ring. The fibrillar orientation of the S2 layer varied alternatively within the range of about 40-45°. It is clear from Fig. 6 that the changes in the orientation angle of helical thickenings nearly parallels those of the microfibrillar angle in the S2 layer. It may be considered that the beginning of the increase or decrease in the fibrillar angle of the S2 layer is a trigger which causes the shifting of the orientation of the helical thickenings because the shifting of the spirals in a series of differentiating cells begins with the stage of the S2-deposition[11].

3.3 Nature of helical thickenings in normal and compression wood

Except for the lack of cavities and the presence of thickenings, compression wood tracheids of *T. cuspidata* had all the other structural characteristics typical of such cells. In normal wood tracheids, helical thickenings were attached loosely on the S3 layer and were oriented as an S-helix in parallel with microfibrils of the S3 layer. Conversely, they were oriented in a Z-helix at an angle of about 45° which was about the same angle as that of the S2 layer. The turn in the direction of the thickenings always occurred in the transition from normal to compression wood, and *vice versa*, and corresponded to the changes of the stimulus of inclination as shown in Figs. 5 and 6. The disappearance or reappearance of the S3 layer and the shifting of the orientation of the thickenings begin nearly in parallel with the transition between normal and compression wood. Also, as

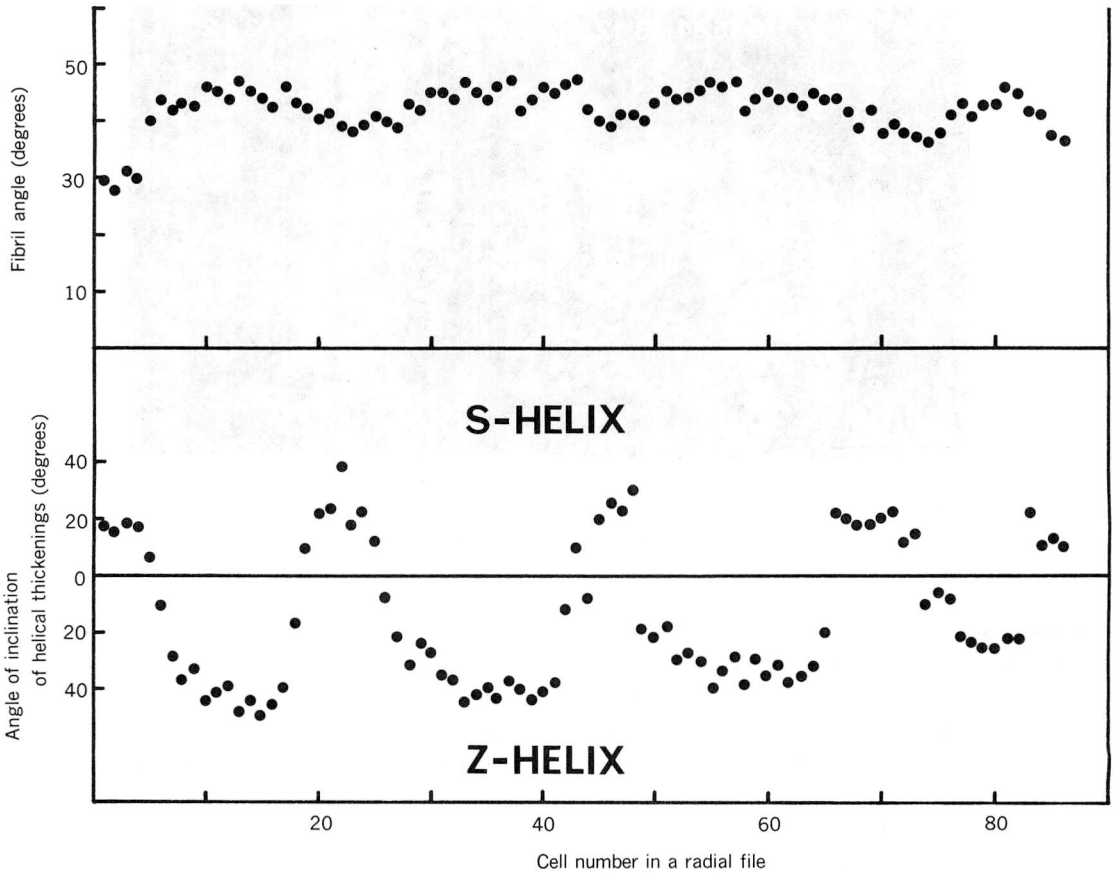

Fig. 6. Changes in the orientation angle of helical thickenings and the fibrillar angle of the S2 layer within an annual ring.
Note: the turns in the direction of helical thickenings correspond to the changes of the stimulus of inclination.

stated above, the shifting seems to occur in the cells at the stage of the S2-deposition. In the present study, certainly in many cases, helical thickenings were found to have the same orientation as the innermost microfibrils of the secondary wall layers. The change in the orientation angle of the thickenings almost paralleled that of the fibrillar angle of the S2 layer in compression wood. However, the orientation of the thickenings often was distorted, being crossed with the innermost microfibrils especially in some tracheids without the S3 layer transition from compression to normal wood. The disappearance and branching of the thickenings also were observed in the transitional zone as shown in Figs. 5 and 7. The fact that helical thickenings are oriented in a crossing pattern with microfibrils of the S2 layer suggests that a shifting of the microtubular orientation occurred during the stage of helical thickening-deposition, after the microfibrils of the S2 layer were deposited, because the microtubules are oriented at the same angle as the cellulose microfibrils of the S2 layer in the S2-deposition stage[17].

In some recent investigations[6][10], thickenings laid on the S2 layer with helical cavities were observed in the transitional tracheids going from compression to normal wood in *Pseudotsuga*, although helical cavities and thickenings are thought to be mutually exclusive in compression wood[9]. This indicates that the orientation of helical thickenings does not coincide always with that of the innermost microfibrils of the secondary wall layers as shown in Fig. 7. The simultaneous occurrence of helical cavities and

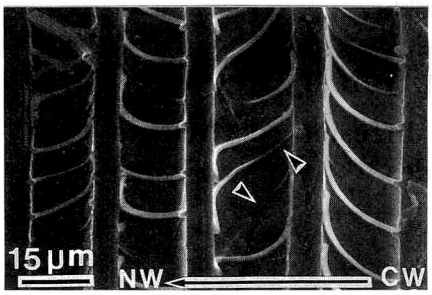

Fig. 7. A scanning electron-micrograph of the inner surfaces of transitioning from compression(CW) to normal wood(NW).
Note: Arrow heads indicate the branched thickenings crossing the innermost microfibrils where the S3 layer is absent.

thickenings in one and the same tracheid is very interesting in relationship to the changes in the protoplast occurring in the developing compression wood tracheids[18]. It may be considered that the complex morphogenic factors involved in the development of the cell wall govern the orientation of helical thickenings.

There is no agreement at present concerning the reversion of helical thickenings. More information on this point is needed to clarify the nature of helical thickenings.

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